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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/175,683	10/20/98	CHEN L	10275-134001

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EXAMINER	
SCHNIZER,R	
ART UNIT	**PAPER NUMBER**
1632	23

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/175,683	Applicant(s) CHEN ET AL.	
	Examiner Richard Schnizer	Art Unit 1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 July 2001* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *6-8,10,20,31-37 and 48* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *6-8,10,20,31-37 and 48* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

An amendment was received and entered as Paper No. 20 on 7/9/01. Claims 1-5, 9, 11, 17-19, 21, 23-30, and 38-47 were canceled, and claim 48 was added as requested. Claims 6-8, 10, 20, 31-37, and 48 are pending and under consideration in this Office Action.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

New Matter

Claims 6-8, 10, 20, 31-37, and 48 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims require the use of "mammary gland-specific" codons. The PTO finds no literal support for mammary gland specific codons in the specification., and Applicant has failed to point to any. When an amendment is filed in reply to an objection or rejection based on 35 U.S.C.112, first paragraph, a study of the entire application is often necessary to determine whether or not "new matter" is involved. Applicant should therefore specifically point out the support for any amendments made to the disclosure. See MPEP 2163.06.

Enablement

Claims 35-37 stand rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods of making polypeptides encoded by SEQ ID NO:1, polypeptides identical to those encoded by SEQ ID NO:1 except that they comprise N182Q and N263Q mutations, and fragments of these polypeptides, by providing a non-human transgenic mammal whose genome comprises a nucleic acid encoding the polypeptide or polypeptide fragment, wherein the nucleic acid has been modified by codon optimization, or removal of AUUUA motifs, or both, wherein the nucleic acid is operably linked to a transcription control sequence which causes transcription in a mammary gland of the animal, and wherein the animal secretes the polypeptide or polypeptide fragment into milk of the animal in an amount that is at least 25%, 50% or 100%, greater than the amount observed under the same conditions for animals comprising unmodified nucleic acids encoding these polypeptides or fragments, does not reasonably provide enablement for methods of producing these amounts of any other parasite protein in a transgenic animal. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The invention is a method of making any parasite protein in the milk of a transgenic mammal.

The essence of the invention is the discovery by Applicant that the Plasmodium falciparum MSP-1 protein is not expressed in cultured mammalian cells or in transgenic mice if the gene

encoding MSP-1 is not modified from its native state. Applicant has shown that specific sequence modifications which increase the GC-content of an MSP-1 transgene, and which also remove the AUUUA motifs from the gene, result in expression of the encoded protein in cultured mammalian cells and in the milk of transgenic mice.

The prior art teaches that codon optimization and/or removal of AUUUA motifs can lead to improved expression of heterologous proteins in mammalian expression systems. See Dziegiel, Seed, and Bosch below under 35 USC 103 rejections. However, the prior art also teaches that the amount to which protein expression can be improved by such modifications is unpredictable. It is assumed in the art that the use of codons corresponding to abundant charged tRNAs results in faster translation and fewer stalled ribosomes. This makes mRNAs poorer targets for RNases, thereby stabilizing them. See Seed (1998) column 1, lines 15-24. It si also known that AUUUA motifs can destabilize mRNAs depending on the presence in the cell of the appropriate destabilizing proteins which bind to these sequences. AUUUA motifs have been shown to be active when located either in the coding region or the 3'-untranslated regions of transcripts. See Akashi et al (1994), abstract and Fig. 1. However, as Applicant points out in Paper No. 16, Akashi also teaches that there are other factors unrelated to AUUUA sequences which play a role in mRNA destabilization. In support of this Liebhaber (1997) teaches that primary, secondary, and tertiary structures of mRNAs influence their stability by determining their exposure to nucleases, However, Liebhaber also notes that the specific higher order structural determinants of mRNA stability were unknown at the time the invention was filed. See abstract.

Applicant has shown that the invention can be used to successfully increase the expression of MSP-1 protein in mammalian cells. Evidence is disclosed which strongly suggests that the invention functions to stabilize MSP-1 mRNA. For this reason, the invention should be useful in increasing the expression of proteins in situations where the primary hindrance to expression is unstable mRNA, assuming that the instability of the mRNA owes to the presence of AUUUA motifs or degradation due to slow translation. However, as discussed above, Akashi and Liebhaber teach that the factors controlling mRNA stability are not limited to the presence of AUUUA motifs and the rate of translation, and can involve secondary and tertiary structures of the message. Because the higher order structural determinants which affect mRNA stability have not been determined and remain under study, one cannot predict a priori how much a given sequence change will improve the stability of a given mRNA, and how much of an improvement in protein production will result. Rather one must examine each case individually, and determine empirically what are the effects of each mutation. One might argue that it is not undue experimentation to determine the degree of stabilization which can be conferred by each particular sequence modification. However as set forth in *In Re Fisher*, 166 USPQ 18(CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> that the scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to **known scientific laws**; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with the degree of unpredictability of the factors involved.

Emphasis added. Because the nature and identity of mRNA destabilizing sequences is highly unpredictable, and the specification fails to provide any means to predict which sequences will destabilize mRNAs, and by what amount, one of skill in the art would have to perform undue experimentation in order to obtain predictable amounts of increased protein expression by use of the claimed methods.

Applicant's response does not address this ground of rejection.

Applicant is encouraged to limit the claims to methods of producing MSP-1.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 6-8, 10, 20, 31-37, and 48 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 6-8, 10, 20, 31-37, and 48 are indefinite because they recite the phrase "mammary gland-specific codon". There is no such thing as a "mammary gland-specific codon" because there are no codons which are specific to mammary glands only. Applicant argues that this term is consistent with the art recognized term "preferred codon". Applicant relies for support on Kotula et al (1991) and Urdea et al (1983), which deal with gene expression in yeast. In response, the PTO notes that these publications strengthen the basis of the rejection inasmuch as

they each avoid the phrase "yeast-specific" which would imply the existence of codons which are specific to yeast. While there are certain codons which are specific to certain bacteria, the Examiner is unaware of any codons that are specific to yeast, and there is no evidence of record that there are any codons which are specific to mammary gland cells.

Claim 31 is indefinite because it recites "the same codon" without proper antecedent basis.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 6-8, 10, 20, 31-34, and 48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dziegiel (1993), Seed (1998), Akashi (1994), Bosch (1994), and Bleck (1996).

Dziegiel teaches an expression vector comprising a nucleic acid encoding an antigen of *Plasmodium falciparum*. See abstract. The expression vector may be used in mammalian cells for the purpose of producing and isolating the antigen, and may be used to construct transgenic animals for the purpose of producing the antigen. The polypeptide produced may be used as a vaccine. See column 18, lines 54-65, and column 19, lines 61-63. The nucleic acid may be modified by silent mutations which favor the codon usage of the organism in which the nucleic acid will be expressed. See column 20, line 66 to column 21, line 7; and column 21, lines 36-40.

The nucleic acid encoding the antigen is only 30% G+C, and comprises at least two AUUUA motifs within the coding region. See column 16, lines 40-43, and bases 962-966, and 1896-1900 in the sequence bridging columns 13 and 14. Dziegiel does not specifically recommend reducing the AT-content of the nucleic acid, the removal of mRNA instability motifs, or the introduction of "mammary gland-specific" codons.

Seed teaches that codon optimization may be used to increase the expression of foreign genes in mammalian cells. See column 1, lines 8-10; and column 2, lines 7-11. Preferred codons are always those with the highest possible GC-content. See lines 33-37, and Table 1, bridging columns 7 and 8. Seed does not use the phrase "mammary gland-specific codons", however for amino acids A, R, N, Q, H, I, L, K, P, F, and S, Seed teaches that the most preferred codon is the same codon which Applicant chose to use most frequently in reducing the invention to practice. Compare Fig. 3A of the instant application to Table 1 of Seed. For this reason it is concluded that Seed teaches "mammary gland-specific" codons for these amino acids. Seed also teaches avoiding the use of AUUUA motifs in synthetic genes. See column 12, lines 35-37.

Akashi teaches that the function of AUUUA motifs is not restricted to their location within the mRNA. These motifs need not be located in the 3'-untranslated region of mRNAs, and are capable of destabilizing mRNAs even when located in the coding region. See abstract, and Fig. 1

Bosch teaches removal of mRNA instability motifs from nucleic acids which are intended to be expressed in heterologous hosts. Bosch also teaches that codon optimization is advisable. See column 4, lines 12-21.

Bleck teaches a method of expressing recombinant proteins in the milk of female mammals. The method comprises operatively linking an alpha-ovalbumin promoter to an exogenous gene sequence and generating a transgenic animal comprising the hybrid gene. The alpha-lactalbumin promoter directs expression in mammary tissue. The expressed protein is secreted into the mammal's milk, from which it can be purified. See column 2, lines 34-46. The transgenic animals may comprise the construct in their germ line cells. See column 9, line 49 to column 10, line 31, especially column 10, lines 29-31.

It would have been obvious to one of ordinary skill in the art at the time of the invention to optimize the codon usage of the transgene of Dziegiel as taught by Seed. One would have been motivated to do so because Seed teaches that codon optimization can improve expression of foreign genes in mammalian cells. One would have been motivated to decrease the AT-content of the transgene because it is apparent from the teachings of Seed that the most preferred codons in mammalian systems are the most GC-rich codons. Similarly one would have been motivated to remove AUUUA motifs from the transgene because Seed teaches that this should be done. Furthermore, it would be inherent in the process of selecting GC-rich codons. For example, depending on the reading frame, the sequence AUUUA can comprise an AUU codon encoding I, a UUU codon encoding F, or a UUA codon encoding L. The preferred codon for each of these

amino acids, as taught by Seed, comprises a G or C. Thus if one followed the teachings of Seed in terms of codon selection, one would necessarily remove AUUUA motifs from the transgene open reading frame. One also would have been motivated to remove AUUUA sequences from the transgene open reading frame because Akashi teaches that AUUUA sequences in open reading frames can destabilize mRNA. One would have been motivated to produce the parasite protein in the milk of the transgenic animal because Bleck teaches that transgenic recombinant polypeptides can be produced in the milk of transgenic animals, and that the produced proteins can be non-native to the animal, and can be used industrially. See column 4, lines 51-57. The obviousness of optimizing codon usage and removing AUUUA sequences from genes intended to be expressed in heterologous organisms is underlined by Bosch who suggests both of these practices.

Thus the invention as a whole was prima facie obvious.

Response to Arguments

Applicant's arguments filed 7/9/01 have been fully considered as they apply to the rejection above but they are not persuasive.

Applicant argues at pages 14 and 15 of the response that Dziegiel teaches none of the elements of the instant invention. On the contrary, Dziegiel teaches a transgenic mammal capable of producing a parasite protein, and suggests that the codons of the transgene should be optimized for expression in the mammal. The elements of the invention not taught by Dziegiel are taught by Seed and Bleck. Applicant also argues that Dziegiel provides no motivation to modify

the a parasite protein to obtain its expression in the milk of a transgenic mammal. Applicant's attention is directed to column 21, lines 36-51 which teaches that codon usage may be modified to correspond to that of the organism into which the nucleic acid is inserted. Because the teachings of Seed indicate that codon optimization for mammalian cells effectively means increasing GC-content, one of ordinary skill in the art would clearly be motivated to increase the GC-content of a transgene which was intended to be expressed in a mammal. Applicant argues at page 15 of the response that the cited references have no teaching or suggestion that codons used in transgenes intended to be expressed in mammals should be selected based on mammary tissue codon usage. This is irrelevant because the claims do not require selection of such codons. The claims require "mammary gland-specific" codons. The PTO maintains the position that there is no such thing as a "mammary gland-specific" codon, because there are no codons which are used only in mammary glands. Applicant has failed to provide any evidence or argument to the contrary. In any case, Applicant has failed to show that Seed does not suggest use of codons which are preferred in messages encoding proteins which are secreted into milk. The PTO reiterates that most of the codons used by Applicant in reducing the invention to practice are the same as those which are used by Seed. See rejection above.

Applicant argues at page 15 of the response that there is no motivation to use the teachings of Seed to arrive at Applicant's invention because Seed does not suggest that naturally occurring parasite proteins would not be expressed in mammalian cells. The PTO believes that Applicant intended to refer to naturally-occurring parasite nucleic acids. Again, Applicant's

attention is directed to column 21, lines 36-51 of Dziegiel which teaches that codon usage in the parasite nucleic acid may be modified to correspond to that of the organism into which the nucleic acid is inserted. Why would Dziegiel suggest codon optimization if there was no motivation to improve expression of the protein? Applicant has provided no reason or evidence why one of ordinary skill in the art would not follow the codon optimization teachings of Seed when making the transgenic animal taught by Dziegiel.

At page 16 of the response, Applicant argues that Bosch's general disclosure of modifying a bacterial protein does not teach the claimed invention. In response, the PTO notes that the rejection would stand without Bosch. Bosch simply provides evidence of that codon optimization and removal of AUUUA motifs are generally-used techniques when expressing genes in heterologous organisms.

At pages 16 and 17 of the response Applicant argues that the teachings of Akashi are insufficient to provide motivation to remove AUUUA motifs from the coding regions of transgenes. This argument is supported by a statement from Akashi to the effect that there may be an alternative explanation for their results. However, one must view this statement, at page 3185, column 2, in the context of the rest of the paper and the teachings of the art. First, Akashi immediately de-emphasizes this possibility by noting that the experiments were internally controlled and that an AT-containing transcript was degraded more rapidly than the corresponding GC-containing transcript. See page 3185, column 2, second full paragraph. Second, one cannot ignore the abstract of the document which states "the AUUUA sequences

when placed in an exon can still modulate the stability of RNAs". If the authors considered this to be an unlikely explanation of the data, it is doubtful that it would have been set forth in the abstract of the paper. Third, the prior art, including Seed and Bosch, teaches that AUUUA motifs should be removed from sequences which are to be expressed in heterologous hosts.

Applicant argues that it is improper to use Applicant's own work as a road map in constructing the invention. This argument is unpersuasive in view of the cited art which teaches each element of the invention and provides motivation to combine them for the reasons given above.

Finally, Applicant argues that their results obtained in expressing MSP-1 were unexpected, and therefore not obvious. This is unpersuasive because one of ordinary skill in the art could have combined the teachings of the prior art for the reasons given above, and would have done so with a reasonable expectation of success in view of the general teachings of the art. For example, Dziegiel, Seed, and Bosch all teach codon optimization, and both Seed and Bosch teach the elimination of AUUUA motifs. Please note that claims 35-37, which require specific minimum levels of expression, are not included in the rejection.

For all these reasons the rejection is maintained.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Richard Schnizer, whose telephone number is 103-306-5441. The examiner can normally be reached Monday through Friday between the hours of 6:20 AM and 3:50 PM. The examiner is off on alternate Fridays, but is usually in the office anyway.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Karen Hauda, can be reached at 703-305-6608. The FAX numbers for art unit 1632 are 703-308-4242, and 703-305-3014.

Inquiries of a general nature or relating to the status of the application should be directed to the Patent Analyst Patsy Zimmerman whose telephone number is 703-308-8338.

Richard Schnizer, Ph.D.

ROBERT A. SCHWARTZMAN
PRIMARY EXAMINER